PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : SORENSEN, Marinus B.

Appl. No. : To Be Assigned Examiner: To Be Assigned

Filed : October 18, 1999 Art Unit: To Be Assigned

For : PROTEIN EXTENSIN AND METHOD FOR CYTOTOXIC ENHANCEMENT OF LYMPHOCYTES

Attorney Docket Number: MCG046US

Prior Application Information:

Prov. Appl. No.: 60/104,720

Filed : October 19, 1998

REQUEST FOR FORMAL PATENT APPLICATION

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

Enclosed is a new patent application for filing today. The details regarding this application are as follows.

Title: PROTEIN EXTENSIN AND METHOD FOR CYTOTOXIC ENHANCEMENT OF LYMPHOCYTES

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Attached is an application for patent including specification, claims, abstract of the disclosure, combined declaration and power of attorney, and small entity declaration.

Attorney Docket No. MCG046US

VERIFIED DECLARATION CLAIMING SMALL ENTITY STATUS
UNDER 37 C.F.R. §§ 1.9(f) AND 1.27(c)
FOR SMALL BUSINESS CONCERN

I DECLARE that I am the Director, Officer of New Nordic Danmark APS, that I am an authorized officer in the United States for the small business concern identified below, and that I am empowered to act on behalf of that concern:

NAME OF CONCERN: **New Nordic Danmark APS**
Ny Ostergade 11, 4
DK-4000 Roskilde
Denmark

I declare that the above-identified concern qualifies as a small business concern as defined in Title 13, Code of Federal Regulations, Section 121.3-18, and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when, either directly or indirectly, one concern controls or has the power to control the other, or a third party (or parties) controls or has the power to control both.

I declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, Application Number _____, filed October 18, 1999, entitled:

TITLE: **PROTEIN EXTENSIN AND METHOD FOR CYTOTOXIC ENHANCEMENT OF LYMPHOCYTES**

If the rights held by the above-identified small business concern are not exclusive, each individual, concern, or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. § 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

NAME N/A

ADDRESS _____

[X] SMALL BUSINESS [ ] NON-PROFIT

I acknowledge the duty to file in this application or patent notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 C.F.R. § 1.28(b)).

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18, United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

18-10-1999
Date

Karl Kristian Jensen
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PROTEIN EXTENSIN AND METHOD FOR CYTOTOXIC ENHANCEMENT OF LYMPHOCYTES

I claim the benefit under Title 35, United Sates Code, § 120 U.S. Provisional Application Number 60/104,720, filed October 19, 1998, entitled PROTEIN EXTENSIN AND METHOD FOR CYTOTOXIC ENHANCEMENT OF LYMPHOCYTES.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to a compound which, through biological signals towards lymphocytes, induces development and maturation of lymphocytes and method to control the cytotoxic enhancement of lymphocytes in the intestine. The invention can be used as a therapeutic method to modulate immune responses in the prevention and treatment of a broad variety of disorders including infections and cancers.

2. Description of the Related Art

Epidemiological studies have provided evidence that dietary components in food influence the development of illnesses, e.g., infections and cancers, in human populations. The immune system combines several different strategies in dealing with abnormal cells (cancer) or foreign organisms (viruses, bacteria, parasites). Exposure of blood cells to foreign or abnormal molecules (antigens) stimulates the growth of specialized white cells (B lymphocytes) which produce antibodies. These circulate in the blood or concentrate at mucosal surfaces such as the lungs, the nose, and the intestines, which are prime entry points for invading organisms. The antibodies recognize and bind with high specificity to their target, marking it out for disposal by the scavenger cells of the body. Even in the absence of a specific immune response, these scavenger cells play an

important role in body defenses. Phagocytes and natural killer cells recognize and destroy cancer cells, viruses, and parasites as well as stimulate other components of the immune system. An important aspect of long-term immunity is stimulation of other white cells (T lymphocytes) by antigens. The T cells produce cytokines which increase the effectiveness of scavenger cells. At the same time, the scavenger cells take up antigens and display fragments of these foreign proteins of their surfaces, increasing T cell stimulation.

The intestinal mucosal immune system is adapted to protect the host against potential pathogens. Thus, a complex population of T lymphocytes can be found in the gut-associated lymphoid tissues (GALTs). Lymphocytes are localized in the Peyer's patches, the lymphoid follicles in the colonic mucosa, in the intestinal lamina propria, and above the basement membrane between epithelial cells, i.e., they are lamina propria lymphocytes (LPLs) and intraepithelial lymphocytes (IELs). More than 95 percent of human LPLs have the alpha/beta isotype of the antigen-specific T-cell receptor (TCR). Additionally, CD4- and CD8-positive T cells are present in the laminapropria and in the peripheral blood in a similar proportion. Usually, immature precursors of T lymphocytes from haematopoietic sources are matured in the thymus, i.e., genes that encode the alpha- and beta-chains of their receptors are rearranged. However, extrathymic T cells which differentiate in the intestines or the liver seems to stand at an intermediate position between natural killer (NK) cells and thymus-derived T cells in the phylogenetic development. The ability of the intestine to induce

development and maturation of extrathymic T cells, e.g., LPLs and IELs, makes phylogenetic sense. From an evolutionary point of view, GLATs constitute the first distinct lymphoid tissues in vertebrates, appearing prior to the spleen, the thymus, the bone marrow, and the lymph nodes.

Extrathymic T cells play a role in (a) aging, (2) conditions of malignancy at tumor sites, (3) intracellular infections, (4) pregnancy, (5) autoimmune diseases, and (6) elimination of abnormal self-cells generated by the body itself. Pathways of T cells may increase, accompanying acute thymic atrophy. Intestinal epithelium may induce IEL development without the action of thymic-derived products, or a thymus may promote extrathymic development processes either directly or indirectly. Recent findings give empirical evidence that alternative mechanisms exist for rendering IELs tolerant of normal host tissues. Thus the intestinal epithelium has an intricate and well-refined process for the elimination of autoreactive T cells in a thymus-independent manner.

Pectins are soluble dietary fibers, which are completely metabolized in the gut due to bacterial fermentation. Pectin is a complex mixture of colloidal polysaccharides found in the primary cell walls of dicotyledons (dicots). In vitro test has demonstrated that several polysaccharides contained in pectin have immune stimulating actions. Rhamnogalacturonan enhance the cytotoxic activity of human natural killer (NK) cells and T cells, arabinogalactan activate macrophage and reduce or inhibit metastasis.

In 1994, an <u>in vivo</u> experiment was designed in Michigan by Avraham Raz, Kenneth Pienta, and coworkers. Laboratory animals were fed with citrus pectins in the drinking water. The purpose was to see if pectic polysaccharides influenced primary tumor growth and metastasis. Male Copenhagen rats had fast-growing, prostate adenocarcinoma cells injected into their thigh. This resulted in death of control animals within approximately 25 days. However, if the primary tumor was removed before metastasis after about 10 days, control animals could be saved from dying. Interestingly, rats consuming 1.0 percent w/v citrus pectins per day from day four showed a significant reduction in spontaneous lung metastases. Additionally, a significant reduction in the average frequency of metastases per lung was observed. This work was reported in an article by Pienta et al., "Inhibition of Spontaneous Metastasis in a Rat Prostate Cancer Model by Oral Administration of Modified Citrus Pectin," *Journal of the National Cancer Institute*, Vol. 87, No. 5, March 1, 1995.

SUMMARY OF THE INVENTION

The compound comprises the protein extensin or a combination of (1) pectin or polysaccharides found in the pectic molecule and (2) extensin.

The invention relates to a method for using extensin or a combination of pectin or polysaccharides found in the pectic molecule and extensin which is beneficial to health because it can enhance the activity of the immune system.

The invention also relates to a method for preparing a therapeutically effective pharmaceutical for the cytotoxic enhancement of lymphocytes.

DESCRIPTION OF THE INVENTION

Pectin is a complex mixture of colloidal polysaccharides found in the primary cell walls of dicotyledons (dicots). It is characterized by the presence of rhamnose (L-Rhap), galacturonic acid (D-GalpA), arabinose (L-Araf), and galactose (D-Galp). Traditionally, pectin is known for the gellifying properties utilized in industrial and household preparations of jellies, jam, and marmalade. Novel use of pectin includes pharmaceuticals, e.g., barrier antacids, protective barrier between tape and skin, and wound dressings in which the hydrophilic polymer creates a moist environment that is advantageous for a more rapid recovery process. Fibers, e.g., pectins, have a positive role in the human diet. Pectin has a backbone of -1,4-D-galacturonan alternating with rhamnogalacturonan-1 (RG-1). -1,4-D-Galacturonan is composed of about one hundred consecutive -(1 4)-linked D-GalpA residues, and the RG-1 backbone which contains D-GalpA and L-Rhap residues has a degree of polymerization up to about 20. Side chains of arabinan, arabinogalactan, and/or galactan are mostly substituted on O-4 of L-Rhap residues and characteristic glycosidic linkages in side chains have been determined. Three-dimensional computer analysis of the pectic backbone has revealed that it is nearly a linear structure. Side chains, e.g., arabinogalactan-II (AG-II), are connected at almost a right angle to the backbone of RG-1 resulting in a

parsley mill structure. These side chains presumably form a hydrophilic network that can retain water within the matrix of cell walls. It is also possible that neighboring backbones have interlacing side chains that make a strong architecture with many weak hydrogen bonds.

Pectins are a mixture of polysaccharides that have a varying degree of neutralization. Pectinic acids and pectinates contain some ester groups whereas pectic acids or pectates have a negligible amount of ester groups. Esterified D-GalpA prevents an enzymatic cleavage of -1,4-D-galacturonan by fungal endo-polygalacturonase (E.C.3.2.1.15). This poly-1 4- -D-galacturonide glycanohydrolase (endo-PG) catalyzes the hydrolysis of -1,4-bonds in demethylated and deacetylated D-galacturonan. It has been shown that oligosaccharide fragments of D-galacturonan are elicitors that induce plant tissue to synthesize phytoalexins which are toxic compounds to fungi. The complex polysaccharide that is left after an endo-PG digestion is RG-1 which has a major glycosyl composition of L-Rhap, D-GalpA, L-Araf, and D-Galp residues. Polysaccharides can be converted into their monosaccharide constituents using hydrolysis at 121°C for one hour with 2 M trifluoroacetic acid. The loss of sugars is moderate, and derivatization is not needed if the monosugars are separated on a CarboPac PA1 column (Dionex Corp.) and measured by pulsed amperometric detection. Rhamnogalacturonan-1 has been solubilized from suspension-cultured sycamore cell walls (Acer pseudoplatanus), and a molecular weight of approximately 200 kDa has been estimated.

Arabinans are branched polysaccharides composed of -1,5-linked chains of L-Araf residues substituted at O-3, and galactans are -(1 4)-linked polymers of D-Galp residues with some 6-linked D-substituted at O-3, and galactans are -(4)-linked polymers of D-Galp residues with some 6-linked D-Galp residues. Arabinogalactans are grouped into two types. The AG-1 is a -1,4-linked D-Galp backbone substituted through O-3 with side chains of D-Galp residues. This is found in pectins-in seeds, bulbs, and leaves. Apparently, AG-II is more widespread in gymnosperms and angiosperms, in seeds, leaves, roots, fruits, gums, saps, and exudates. It is a very branched polymer that contains a backbone of (1 3)- -linked D-Galp residues with side chains of (1 6)- -linked D-galactooligosaccharides that may have L-Araf residues linked (1 3) or (1 6) to the D-Galp residues. Arabinose residues may be attached to each other by (1 3) and/or (1 5)-links. Plant gums are commercially valuable, and the intensively studied gums from acacia (Acacia senegal) and related species are usually water-soluble polymers. In health care, small arabinogalactans may be candidates for hepatocyte-directed drug delivery.

Extensin is a hydroxyproline-rich glycoprotein (HRGP) particularly abundant in the cell walls of dicots. Extensin contains the amino acids: valine, tyrosine, histidine, threonine, and lysine. Extensin is a rodlike molecule characterized by a polyproline-II helical structure which stabilizes molecular shape and makes hydrogen bond formations possible to adjacent molecules. In the carrot, Araf and Galp residues comprise 65 percent of the weight of an 86 kDa

extensin, 97 and 3 percent, respectively. Hydroxyproline residues comprise 45 percent of the protein, and the proportion of polyproline-II conformation can be determined from circular dichroism studies. Deglycosylation by hydrogen fluoride caused much of the helical secondary structure to be lost. This indicates that the carbohydrates are essential for the native conformation of the protein backbone.

Extensin is synthesized as a soluble monomer and subsequently polymerized into an insoluble polymer in the cell wall. The Golgi apparatus is the site of assembly of glycoproteins. The protein moiety of extensin is about one-third of the total weight, and abundant amino acids are trans-4-L- hydroxyproline to which are attached short carbohydrate side chains: serine, valine, tyrosine, histidine, threonine, and lysine. Repeating motifs are commonly recognized, e.g., Ser-Hyp4 and Val-Tyr-Lys, and similarities exist between different plant species. However, the tetra-hydroxyproline block has not been found in the sugar beet in which the sequence is interrupted: Ser-Hyp2-S-Hyp2-Thr-Hyp-Val-Tyr-Lys. Here X represents an insertion of Val-His-Glu Lys-Tyr-Pro. Apart from this, the sugar beet extensin has a repeating sequence of amino acids analogous to the sequences found in tomato (Lycopersicon esculentum), carrot (Daucus carota L.), and tobacco (Nicotiana tabacum). The hydroxylation of proline residues is a post-translational modification by prolyl hydroxylases (E.C. 1.14.11.2), that may depend on the amino acid sequence in the extensin molecules. Thus, the dipeptide sequences Lys-Pro, Tyr-Pro, and Phe-Pro are not found to be hydroxylated in

contrast to Pro-Val. Complete sequences of extensins are not easily determined because they are usually very insoluble. One approach is to investigate the soluble precursors of extensin, or to screen for extensin in a cDNA library. In dicots, hydroxyproline residues may be O-glycosylated with a single sugar (arabinose or galactose) or up to four Araf residues in an arabino-oligosaccharide. Most of the serine residues, e.g., the Ser-Hyp4 repeats in particular, are O-glycosylated with a single Galp residue. Prolyl hydroxylase appears to be an important enzyme for normal cell morphology. Tobacco protoplasts treated with micromolar concentrations of 3,4-dehydro-L-proline, which is a selective inhibitor of prolyl hydroxylase, developed an abnormal cell wall structure, and cell division was inhibited.

The plant cell wall proteins are divided into five classes. Although differences exist, common features have been identified. Like extensin, the proline-rich proteins (PRPs) are insolubilized in the cell wall, and the relatively high content of tyrosine residues in PRPs can participate in isodityrosine cross-links. The potato tuber lectin (a solanaceous lectin) has a serine-hydroxyproline-rich glycopeptide domain that resembles extensin, and this can also accumulate in response to wounding. Solanaceous lectins are apparently associated with cell membranes instead of cell walls, and they are glycosylated the same way as extensin. Glycine-rich proteins (GRPs) may contain up to 70 percent glycine residues arranged in short repeats, and GRPs are like extensin expressed in response to a variety of developmental and stress conditions.

Tissue-specific expression of extensin has been examined by immunolocalization in the light microscope in leaves, stems, roots, fruit, and tuber of carrot, tomato, and potato, and all cells displayed a varying degree of staining. The functions of extensin have focused attention to developmental control, defense against various pathogens, e.g., fungi, bacteria, and viruses, and wound healing. Wounding induces a rapid activation of a specific class of serine threonine protein kinases, and this suggests the presence of an intracellular signal transduction pathway related to the wound stimulus. Moreover, extensin may link covalently to other cell wall macromolecules, e.g. arabinogalactan and rhamnogalacturonan-1 (RG-1). Evidence for a covalent cross-link between extensin and RG-1 has come from the studies of cell walls from cotton (Gossypium hirsutum L.). Cell walls of suspension cultures were subjected to endo-polygalacturonase, cellulase (endo- -1,4-D-glucanase, E.C. 3.2.1.4), anhydrous hydrogen fluoride solvolysis, ammonium bicarbonate extraction, and trypsin (E.C. 3.4.21.4). After this treatment, only sugars indicative of RG-1 and extensin remained in soluble.

Following in vitro testing of polysaccharides found in pectin, we have done scientific <u>in vitro</u> experiments to test synergistic effects of pectic molecules and extensin. We identified that a combination of pectic molecules with the protein extensin containing valine, tyrosine, histidine, threonine, and lysine can activate eycarotic [eukaryotic?] cells in significantly smaller concentrations than pectic molecules alone. A combination of pectin with extensin in a ratio higher than seen in natural plant cells can be used as a

therapeutic method to modulate immune responses in the treatment of a broad variety of disorders including infections and cancers.

In summary, the present invention relates to the protein extensin, a combination of compounds which comprise (1) extensin, or (2) a combination of pectin or polysaccharides found in the pectic molecule and extensin, being able to induce development and maturation of extrathymic lymphocytes.

The invention can be used as a method to enhance the cytotoxic activity of lymphocytes in relation to conditions of malignancy at tumor sites, intracellular infections, autoimmune diseases, and elimination of abnormal self-cells generated by the body itself.

The invention was the result of a laboratory test program using in vivo cell signalling model. The application of a nutritionally complete synthetic (NCS) medium in an oligo-cell experimental research program in which cells demise (or eventually have a prolonged lag-phase) was used for gaining scientific information related to determining the effectiveness of pectins and pectin and extensin combinations as biochemical messengers. An additional control was supplemented with cephalin, that activates cells to multiplication. The effect of an activator added to the NCS medium is compared directly to the control that demises. Apparently, any compound or combination of compounds can be examined in light of the question whether it can activate a cell to survive and/or proliferate. From the beginning of the 1980's the effect of biochemical messengers has been examined with cells grown in an NCS medium free of proteins, lipids,

and sugars. Signalling applies to unicellular and multicellular organisms, and the idea of NCS media is that a biochemical messenger can be tested for its biological effect on the cells to gain insight in cellular signalling mechanisms. Biochemical messengers are related to the four biological cornerstones of the eukaryotic cell: survival, proliferation, differentiation, and programmed cell death.

In oligo-cell experiments, different pectins and combinations of pectins and extensins were examined for their ability to activate Tetrahymena thermophila cells to survival and/or proliferation. The Tetrahymena cell has nutritional requirements similar to those of animals. They contain subcellular organelles such as mitochondria and peroxisomes, which are essential for the development of regulatory mechanisms in cellular metabolism. Although, apparent differences in phylogenesis exist between unicellular species and multicellular species, it is of interest that they have similarities, which justify the use of Tetrahymnea as a substitute for mammal cells in this investigation, e.g., biochemical messengers, signal transduction pathways, or resemblance of structural components. Two examples are the T. thermophila ribosomal protein S/ which is homologous to mammalian ribosomal protein S4, and the carbohydrate metabolism which is closely related to that of mammals. Glycogenesis, glycolysis, tricarboxylic acid cycle, and lipid metabolism have been reviewed. Cloning efficiencies were evaluated after 30 hours and compared to the controls that were supplemented with cephalin which sowed cloning efficiencies of 92 percent. When a combination of pectins and

extensin was added T. thermophila cells were activated, and the best survival frequency was demonstrated. None of the other compounds tested activated T. thermophila.

Example
Pectin Containing Extensin

This experiment was set up to evaluate the effectiveness of different types of pectins with different content of proteins (extensin) and a partially purified extensin.

Oligo-cell experiments with Tetrahymena thermophila

Composition of medium	mg/L	Cloning efficiencies
Synthetic nutrient medium		0/15
cephalin	50	15/15
extensin partially purified from sugar beet pectin	25	12/15
citrus pectin	25	3/15
citrus pectin saponificated	25	3/15
beet pectin	25	8/15
beet pectin saponificated	25	1/15
apple pectin	25	4/15
apple pectin saponificated	25	1/15

The initial cell concentration was about two cells in a two-mL volume.

Cloning efficiencies were determined from cell multiplications in a synthetic nutrient medium without and with supplements. The first number given was the number of experiments that had multiplication of cells after 30 hours. The second number given was the total number of experiments.

In the above experiment the partially purified extensin showed cloning efficiencies of 80 percent. The extensin was

partially purified from sugar beet pectin (Beta vulgaris). The partly purified extensin can be described as rhamnogalacturonan proteins containing non-covalent bonds between hydroxyproline-rich glycoproteins and rhamnogalacturonan-1.

Native nonsaponificated pectin is apparently more effective than saponified pectin. The data suggest that pectins with a higher protein (and extensin) content are more effective than pectins with lower protein content.

As the purified extensin is difficult and expensive to commercialize in larger scale, this experiment aimed to see if similar effects could be obtained by protein (extensin) rich fibers from sugar beet alone or if there would be a synergistic advantage of combining pectin with protein/extensive rich fibers from sugar beet.

Oligo-cell experiments with tetrahymena thermophila

Composition of medium	mg/L	Cloning efficiencies
Synthetic nutrient medium		0/15
cephalin	50	15/15
extensin partially purified from sugar beet pectin	25	11/15
sugar beet fiber	25	11/15
50% citrus pectin/ 50% sugar beet fiber	25	3/15
50% beet pectin/ 50% sugar beet fiber	25	15/15
50% apple pectin/ 50% sugar beet fiber	25	10/15

The initial cell concentration was about two cells in a two-mL volume.

Cloning efficiencies were determined from cell multiplications in a synthetic nutrient medium without and with supplements. The first number given was the number of experiments that had multiplication of cells after 30 hours. The second number given was the total number of experiments.

CLAIMS

What is claimed is:

1. A compound comprising a protein of extensin or a combination of (a) pectin or polysaccharides found in the pectic molecule and (b) extensin.

2. A method for cytotoxic enhancement of lymphocytes comprising ingesting a therapeutically effective amount of extensin or a combination of pectin or polysaccharides found in the pectic molecule and extensin to enhance the activity of the immune system.

3. A method for preparing a therapeutically effective pharmaceutical for the cytotoxic enhancement of lymphocytes comprising combining a therapeutically effective amount of extensin or a combination of pectin or polysaccharides found in the pectic molecule and extensin to enhance the activity of the immune system with an excipient.

ABSTRACT OF THE DISCLOSURE

The invention is a compound and method for enhancing the immune system. The compound is extensin or a combination of (a) pectin or polysaccharides found in the pectic molecule and (b) extensin. The method for cytotoxic enhancement of lymphocytes requires ingesting a therapeutically effective amount of extensin or a combination of pectin or polysaccharides found in the pectic molecule and extensin to enhance the activity of the immune system. The invention also relates to a method for preparing a therapeutically effective pharmaceutical for the cytotoxic enhancement of lymphocytes.

DECLARATION

AS A BELOW NAMED INVENTOR, I declare that:

My residence, post office address, and citizenship are as stated next to my name. I believe that I am the original inventor, of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE: **PROTEIN EXTENSIN AND METHOD FOR CYTOTOXIC ENHANCEMENT OF LYMPHOCYTES**

the specification of which either is attached or otherwise accompanies this Declaration, or

[x] was filed in the U.S. Patent and Trademark Office on Oct. 18 , 1999 and assigned Serial No. _____,

[ ] and (if applicable) was amended on _____,

[ ] was filed as a PCT international application on _____ and assigned No._____,

[ ] and (if applicable) was amended under PCT Article 19 on _____,

I state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56. I further acknowledge, in the case of any application filed pursuant to Title 35, United States Code, § 120 (and which discloses and claims subject matter in addition to that disclosed in the prior copending application), the duty to disclose all information known to the persons to be material to patentability as defined in 37 C.F.R. § 1.56 which information became available between the filing date of the prior application and the national or PCT international filing date of the subject 35 U.S.C. § 120 application.

I claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventors' certificate listed below and have also identified below any foreign application for patent or inventors' certificate having a filing date before that of the application on which priority is claimed:

N/A

Yes [ ] No [ ]	*(Application Number)*	*(Country)*	*(Day/Month/Year filed)*

I claim the benefits under Title 35, United States Code, § 120, of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a), which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

60/104,720	10/19/98	Pending
(Application Serial No.)	*(Filing Date)*	*(STATUS: patented, pending, abandoned)*

I appoint the following attorneys, Paul Grandinetti, Reg. No. 30,754 and James L. Lewis, Reg. No. 24,732 to transact all business in the U.S. Patent and Trademark Office connected therewith and with any divisional, continuation, continuation-in-part, reissue, or reexamination application, with full power of appointment and with full power to substitute an associate attorney or agent, and to receive all patents which may issue thereon. I request that all correspondence be addressed to:

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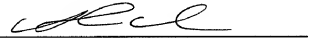
I DECLARE that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

INVENTOR: Marinus Blaabjerg Sorensen Citizenship: Denmark

Inventor's signature:  Date: 6th October 1999

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